

# New light on prions: putative role of co-operation of PrP<sup>C</sup> and A $\beta$ proteins in cognition

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## Abstract

A seminal article of Takahashi *et al.* reporting concomitant accumulation of cellular prion protein (PrP<sup>C</sup>) and  $\beta$ -amyloid (A $\beta$ ) in dystrophic neurites, within neuritic plaques raised an exciting issue that is important for our understanding of mechanisms of neurodegeneration. The mentioned authors interpreted their findings rather cautiously, however since the time of their publication, several reports representing different approaches and methods have seemed to indicate that both proteins appear to co-operate more intrinsically than it could have been imagined earlier. The goal of the review is to sum up the accruing research data with special attention to evidence pointing to the co-operative role of PrP<sup>C</sup> and A $\beta$  in cognitive impairment.

**Key words:** prion protein,  $\beta$ -amyloid, cognitive impairment, Alzheimer disease, tau protein.

## Introduction

Though Alzheimer's disease (AD) and Creutzfeldt-Jakob disease (CJD) are characterized by distinct neuropathological changes, they share common pathological features. They are both conformational diseases, related to accumulation of altered proteins, which results in a loss of global cognitive functions. Alzheimer's disease is a predominant neurodegenerative disorder characterized by two major pathological changes: amyloid plaques and neurofibrillary tangles. Amyloid plaques are extracellular formations consisting of  $\beta$ -amyloid (A $\beta$ ) and cellular material outside and around neurons. Neurofibrillary tangles are intracellular aggregates of microtubule-associated tau protein, which has become hyperphosphorylated and misfolded. Creutzfeldt-Jakob disease is

a rapidly progressive brain disease caused by infectious-like self-perpetuating mechanism leading to conversion of physiological cellular prion protein (PrP<sup>C</sup>) to its Scrapie conformation (PrP<sup>Sc</sup>) [52]. PrP<sup>Sc</sup> creates extremely stable forms, which accumulate in infected tissue resulting in its spongiform degeneration [53]. There are little data concerning interactions between prion proteins and A $\beta$ , both in their physiological and conformationally changed form with regard to cognitive functions and dementia. Takahashi *et al.* reported concomitant accumulation of PrP<sup>C</sup> with A $\beta$  in dystrophic neurites within one of the amyloid plaque types, called neuritic plaques [82]. Also A $\beta$  was found to be deposited with PrP<sup>Sc</sup> in CJD [26]. Due to the suspicious findings of those proteins within the same individual, speculations arise

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around the possibility of connection between the pathophysiological processes occurring in these two neuropathological conditions [9,70]. These interactions may also have an impact on cognitive performance. Therefore, the following question arises: do prions and A $\beta$  co-operate in cognitive impairment? Here, we review recent findings at the crossroads of cognitive neuroscience and neuropathology in order to expose the independent role of these proteins in cognition and their possible interactions, and to seek the answer to this question.

## Prion protein

PrP<sup>c</sup> is a highly conserved protein, which may be found in most of vertebrates, at every stage of their development and in all types of tissues, especially in the nervous system [79]. PrP<sup>c</sup> is a glucolipid-anchored cell membrane sialoglycoprotein, which is localized in raft-like microdomains [57]. It is presented on the presynaptic and postsynaptic membranes [91] of neurons, of many brain areas including hippocampus and the cortex [73]. Membrane-anchored PrP<sup>c</sup> passes internalization and recycling through the endosomal pathway [58]. It becomes internalized and degraded in lysosomes [68] or is released into the extracellular space [77]. Proposed roles in the physiology of this protein are related to its localization on the cell surface. PrP<sup>c</sup> may act as a cellular adhesion molecule which plays a part in neurite outgrowth, neuronal differentiation and survival [54]. These functions are consistent with a finding that PrP<sup>c</sup> can associate with surface proteins like laminin, laminin receptor precursor, and the neural cell adhesion molecule (NCAM) [6]. Animal studies have also revealed that absence of PrP<sup>c</sup> is connected with disrupted olfactory physiology and behaviour [79], altered circadian clock, modification of sleep pattern [37], and increased sensitivity to seizures [85]. New reports bring new data indicating various potential features of this protein, making it difficult to find its one consistent function.

A growing number of works on mice models and on humans indicate a possible role of prion protein in the area of cognitive functions. The most common source of those reports comes from mice models of PrP<sup>c</sup> gene ablation or over-expression. PrP<sup>c</sup> null mice presented hippocampus-dependent spatial learning and memory consolidation impairment [23]. Interestingly, those changes were reversed by

re-expression of PrP<sup>c</sup> [12]. Additionally, deficiency of this protein may cause attention deficits [56]. Knockout mice were also more vulnerable to age-related decline in memory [20] and motor processes [64]. Moreover, PrP<sup>c</sup> over-expression leads to hyperactivity, increased preference for visual, tactile and olfactory stimuli associated novelty [73]. Also it is noteworthy that a five-fold increase in PrP<sup>c</sup> expression in comparison to wild type mice results in an increased resistance against age-related cognitive decline [72]. Due to the plenty of PrP<sup>c</sup> functions there are many possible molecular mechanisms in which this protein affects cognitive processes. PrP<sup>c</sup> is localized in the synaptic area, and it may interact with other proteins in this structure, playing a role in the synaptic plasticity [91]. An *in vitro* study has revealed that recombinant PrP (rPrP) induces rapid processing of both axons and dendrites as well as it increases the number of new synapses [40]. Consistently, deletion of PrP<sup>c</sup> resulted in impaired LTP together with a decrease in fast GABA-A receptor-dependent inhibition [21,22]. Perhaps changes in LTP are associated with the ability of PrP<sup>c</sup> to make physical interaction with receptors for the glutamate. It has been shown that PrP<sup>c</sup> is able to co-immunoprecipitate with NR2D subunit of the NMDA, suggesting its possible direct modulation [6]. Transgenic (Tg) mice lacking PrP<sup>c</sup> showed enhanced NMDA-induced currents, which became reversed after its over-expression [42]. PrP<sup>c</sup> also affects kainate receptors [71] and metabotropic glutamate receptors [4] suppressing neuronal excitability through many different mechanisms. Noteworthy, it has been proved that the range of synaptic responses increased with the (higher) level of the PrP<sup>c</sup> expression within glutamatergic synaptic transmission in the hippocampus, but the overall probability of transmitter release appeared unchanged [12]. This example shows that PrP<sup>c</sup>-dependent modulation of glutamatergic stimulation and plasticity is more complex than just the control of the level of neurotransmitter. Regulation of glutamate signalization within synapses is important not only because of plasticity, but also due to the fact that abnormal Ca<sup>2+</sup> currents, caused by aberrant activation of the NMDA receptor, results in excitotoxicity [32], which leads to neurodegeneration. Experiments mentioned above indicate that PrP<sup>c</sup> modulates this process. This neuroprotective mechanism may be the second way in which PrP<sup>c</sup> is involved in cognitive functions. Tg mice expressing modified PrP (with-

out its central region residues 105-125 –  $\Delta$ CR PrP), resulted in massive excitotoxic degeneration of cerebellar granule neurons [15,51]. There is evidence that PrP<sup>c</sup> also plays a role in neuroprotection through the regulation of intracellular signalling cascades, mediating cellular survival [55], and its over-expression protects cell lines from apoptosis. Protective activity has been also proved in glia cells. PrP<sup>c</sup> acted as a radical scavenger in both ROS-rich solution and astrocytes cultures *in vitro*, and its activity was essential in their protection against oxidative stress. This feature may reflect its protective functions in conditions similar to those observed during neurodegeneration and ischemia [5]. Another defensive mechanism is associated with the ability of PrP<sup>c</sup> to bind co-chaperon molecule, called stress-inducible protein 1 (STI1). They create a complex that acts as a survival and differentiation promoter [88]. Intriguingly, blocking the connection between PrP<sup>c</sup>, STI1 and laminin adversely influences memory [18,19]. There are only scarce data indicating cognitive functions of PrP<sup>c</sup> in humans, but they bring promising results. One epidemiological study of 1322 elderly participants revealed that subjects in higher serum PrP<sup>c</sup> quintiles appeared to have lower cognitive functioning scores than those in the lowest PrP<sup>c</sup> quintile. There are two proposed mechanisms of serum PrP<sup>c</sup> elevation. Either there is reduced nerve cell integrity, or a higher serum PrP<sup>c</sup> level reflects the abundance of PrP<sup>c</sup> in neuro-cellular membranes [8].

Exploratory analysis of 335 healthy volunteers revealed that even SNP of the PrP<sup>c</sup> gene might influence cognitive functions in humans, especially a common polymorphism at codon 129, which results in the translation of methionine or valine on a short  $\beta$ -sheet region in the C-terminal domain of the protein [77]. Methionine at codon 129 is associated with lower scores on several subscales of HAWIE-R subscales (German version of the Wechsler Adult Intelligence Scale Revised), especially with the Digit Symbol subtest. Interestingly, PrP-IQ association was the strongest in the less educated individuals; as opposed to other studies showing that the genetic influence on IQ is higher among higher educated families [76]. The same polymorphism is associated with the reduction of white matter in a group of healthy volunteers and patients with schizophrenia [78]. However studies on long-term memory revealed that healthy subjects presenting the same Met129 yielded the highest effect size, recalling

17 percent more words twenty four hours after the list-learning task than carriers of Val129 gene type, but there was no significant difference between those groups in short-term memory. Authors of this study hypothesize that despite the fact that Met129 allele may facilitate self-perpetuating conformational changes of the human prion protein, it may have a beneficial effect on long-term memory by hypothetical prion-based mechanism [66]. Studies mentioned above revealed that PrP<sup>c</sup> and its gene may aspire to the role of a potential biomarker of cognitive measurements. The scarcity of investigations in humans and a plenty of possible mechanisms limit possibilities to draw a definite conclusion as for the existence of one causative relation between PrP<sup>c</sup> and cognition. In spite of divergence of its functions, subserving somewhat unrelated processes, there is a prospect to indirectly indicate common ground of its activity. PrP<sup>c</sup> may act as a protein, involved in global protection of the organism in a micro- and macroscopic perspective: at the microscopic (cellular) level – protecting the cell against apoptotic factors, ROS, excitotoxicity, toxins and at a macroscopic level – affecting the whole organism, by playing a significant role in cognition, especially in defensive attention, spatial and long-term memory and also olfactory physiology and behaviour (crucial for the survival chances of an animal).

## $\beta$ -amyloid

$\beta$ -amyloid is a peptide consisting of 36-43 amino acids which originates from the cleavage of the transmembrane glycoprotein called amyloid precursor protein (APP), by the proteolytic activation of  $\alpha$ -,  $\beta$ - and  $\gamma$ -secretase [80]. Generation of A $\beta$  may occur in the neuronal axonal membranes and is preceded by APP-mediated axonal transport of  $\beta$ -secretase and presenilin-1 [39]. Amyloid precursor protein is cleaved by  $\beta$ -secretase, producing soluble and a cell-membrane bound fragment of APP [46], which is then cleaved by  $\gamma$ -secretase. This reaction produces APP intracellular domain (AICD) associated with the regulation of gene transcription and A $\beta$ , which is released to plasma, cerebrospinal fluid and brain interstitial fluid [30,90]. It has been established that A $\beta_{39-42}$  are hydrophobic self-aggregating peptides, of which A $\beta_{42}$  is a major component of senile plaques observed in AD, but it still remains controversial how those peptides are involved in the

cognitive decline observed during this disease [36]. The “A $\beta$  cascade hypothesis” suggests the major role of amyloid plaques, especially fibrillar A $\beta$  ones in the aetiology of AD, reporting a correlation between the amount of those formations and cognitive dysfunctions [3,24,25,35,67]. Recent studies with the use of detailed measures of A $\beta$  pathology suggest an opposite explanation. Research on APP<sup>swe</sup>/PS1 $\Delta$ E9 double transgenic mice (well-established model of AD) has revealed that hippocampal soluble A $\beta_{1-40}$  and A $\beta_{1-42}$  levels were highly correlated with spatial learning and long-term contextual memory impairments. Also, hippocampal soluble A $\beta_{1-40}$  and A $\beta_{1-42}$  levels were strongly correlated with spatial memory impairments, but no correlations were observed between mentioned cognitive functions and amyloid plaque formations such as: total A $\beta$  plaque load, fibrillar A $\beta$  plaque load and also insoluble A $\beta$  levels. Authors of this study revealed that a tiny fraction of soluble peptides in the hippocampus and cortex is an independent factor in predicting cognitive impairments in this transgenic mice model, suggesting “soluble A $\beta$  hypothesis” as a major mechanism of cognitive decline in AD [89]. Consistently with this hypothesis, experiments on young domestic chicks show that an injection of soluble A $\beta$  5 minutes prior to training caused memory loss, due to the consolidation failure 35 minutes later [31]. Also, reduction of soluble A $\beta_{42}$  or A $\beta_{42}$  and A $\beta_{40}$  by  $\gamma$ -secretase modulators (GSMs) ameliorated cognitive deficits in Tg2576 plaque-free mice model of AD. However, a later study suggests that newly synthesized soluble A $\beta_{42}$  may play a more significant role in cognitive impairments than plaque-associated soluble A $\beta$  [61]. Injections of A $\beta$  to rats result in rapid cognitive disruption, showing a direct interference with the cognitive functions not only through neurodegeneration, but also through interruption of their cellular mechanisms [17,50,69]. It has been shown that A $\beta$  impairs hippocampal long-term potentiation (LTP) by deterioration of tetanus-induced activation of guanylate cyclase and increase of cGMP. Those changes prevent protein kinase G activation and phosphorylation of GluR1, finally resulting in impaired translocation of AMPA receptors to synaptic membranes [62]. Interactions of A $\beta$  with nicotinic, insulin and glutamatergic receptors may also have an impact on synaptic plasticity and spine formation [17,50,63]. These mechanisms explain why injection of A $\beta$  oligomers before the acquisition of new information disrupts the process

of the consolidation without affecting its retrieval, when the information was properly stored [28]. Furthermore, it has been shown that A $\beta$  may have an impact on the cognitive function through its influence on NADPH oxidase enzyme (NOX) activation. NOX is responsible for production of free radicals, and also it plays a role in neuronal physiology, particularly in hippocampal electrophysiology [43,83]. Data show a significant direct linear relationship between NOX activity, cognitive impairment and age-dependent increase in A $\beta_{1-42}$ . This correlation suggests that oxidative stress caused by NOX-associated redox pathway may be another possible mechanism in which A $\beta$  is involved in cognitive decline [10]. Oxidative stress associated with membranes is a possible mechanism in which A $\beta$  may cause synaptic dysfunction and disruption of cellular ion homeostasis [59]. Interestingly, mounting research show intraneuronal accumulation of A $\beta$  as a possible mechanism of cognitive dysfunction, especially in the early stages of the AD [34,45,87]. This accumulation is associated with morphological alterations of synapses [81] and with a decrease in synaptophysin around the affected neurons in AD patients [38]. Studies on 3xTg-AD mice showed a correlation between the cognitive and synaptic dysfunction with the accumulation of intraneuronal A $\beta$  which occurred before formation of amyloid plaques [7,65]. Also hypercholesterolemia accelerates intraneuronal accumulation of A $\beta$  oligomers, resulting in synapse loss and memory impairment [84]. This view of the complexity of the mechanisms and forms by which A $\beta$  affects cognitive functions will be helpful for proper understanding of its possible interactions with PrP<sup>C</sup>.

## Cooperation?

Tellingly, comprehensive studies have shown that out of 225 000 proteins screened in a cell model, only those with PrP<sup>C</sup> expression were strongly binding soluble A $\beta_{42}$  [49]. It has been proposed that PrP<sup>C</sup> exhibits receptor affinity to  $\beta$ -sheet-rich conformers due to its ~95-110 region and the cluster of basic residues within the N-terminal 23-27 segment [14]. As to the protective functions of PrP<sup>C</sup> mentioned before, one may say that this protein will also protect the cell against A $\beta$ , but a growing number of research comes with opposite findings and also with new controversies. Lauren et al. proposed that synthetic oligomeric forms of A $\beta$  impair LTP through

their interactions with PrP<sup>C</sup> [49], but other studies did not confirm this result [1,11]. However, more novel findings showed that antibodies against 94-104 domain of PrP<sup>C</sup> blocked inhibition of LTP caused by soluble extracts of AD brain [2,29]. Also hippocampal slices lacking PrP<sup>C</sup> were resistant to LTP inhibition by A $\beta$ . Similar relationships can be observed in studies on A $\beta$ -dependent neurotoxicity. Prnp 0/0 mice are more resistant to neurotoxic effects of A $\beta$  oligomers [44] and accordingly, over-expression of PrP<sup>C</sup> in neuronal cell lines increases vulnerability to such effects. It has been also shown that deletion of PrP<sup>C</sup> expression in APPswe/PSen1 $\Delta$ E9 rescues 5-HT axonal degeneration, loss of synaptic markers and early death, and interestingly, Tg mice containing A $\beta$  plaques, but lacking PrP<sup>C</sup> show no spatial learning and memory impairments [33]. APP/PS1 Tg mice, treated for 2 weeks with intraperitoneal injections of 6D11 anti-PrP antibodies, recovered in cognitive learning tasks behaving the same as wild-type mice [16]. Surprisingly, mentioned studies revealed not only that PrP<sup>C</sup> is not neuroprotective against A $\beta$ , but even it may be necessary for its neurotoxicity and its impact on cognitive functions. Nevertheless, some authors have reached opposite conclusions. Rial *et al.* showed that Tg-20 mice characterized by a five-fold increase in PrP<sup>C</sup> expression was resistant to a single intracerebroventricular injection of 400 pmols/mouse of aggregated A $\beta$ <sub>1-40</sub>, revealing no impairments of memory and spatial learning in comparison to the wild type and PrP<sup>C</sup> knockout mice. This resistance was accompanied with a decrease in activated caspase-3 protein and Bax/Bcl-2 ratio and reduced hippocampal cell damage [73]. Calella *et al.* [11] showed that the PrP<sup>C</sup> level had no effect on LTP impairment in APP/PS1 mice, and those results were also confirmed by Kessels *et al.*'s studies [41]. Also participation of PrP<sup>C</sup> in mediation of Ab neurotoxicity had been challenged by results of studies on isolated hippocampal cells from Prnp 0/0 and Prnp +/+ mice. Authors concluded that PrP<sup>C</sup> in specific conditions may exert a relevant role in neurotoxicity because of sequestration of A $\beta$  oligomers rather than a functional activity associated to the protein (for review [28]). A more recent study shed a light on the interactions of these proteins adding some important premise to proper understanding of the controversies and the confounding results mentioned above. Larson *et al.* revealed that AD brain-purified A $\beta$  dimers are specifically binding PrP<sup>C</sup>. This complex

triggers Src Tyrosine Kinase Fyn, which activates the kinase and leads towards abnormal phosphorylation of Fyn and tau. This reaction occurs in neuronal dendritic spines and leads to aberrant tau missorting and hyperphosphorylation. Authors also revealed that dosage of Prnp regulates these changes. This comprehensive study made *ex vivo*, *in situ*, and *in vitro* indicates that this PrP<sup>C</sup>-mediated process may play an important role in late stages of AD, when A $\beta$  dimers reach their highest level [47].

Proper understanding of this finding is facilitated by Chen *et al.*'s study on human neuroblastoma cells. They found that over-expression of PrP<sup>C</sup> downregulates tau protein transcription level through Fyn, Fyn kinase and MEK pathway.  $\beta$ -amyloid oligomers reverse this pathway by binding to PrP<sup>C</sup>, probably by inducing its surface retention that interferes with caveolae-mediated PrP<sup>C</sup> endocytosis and Fyn activation. Phosphorylated Fyn level increased in a dose-dependent manner 2 hours after A $\beta$  oligomer treatment and interestingly it became decreased 1 day after this treatment. Surprisingly, the murine PrP<sup>C</sup> M128V, which correspond with the high AD risk polymorphic human PrP<sup>C</sup> M129V [74] allele was able to bind A $\beta$  oligomers, but it was unable to reverse the tau reduction [13]. This may be another explanation why this polymorphism was associated with lower IQ and white matter reduction in the study mentioned earlier in this article.

The above authors (see ref. [13, 47], and also Larson and Lesne [48]) suggest that confounding results about PrP<sup>C</sup>-mediated impairments, as those described previously, may be attributed only to a subset of A $\beta$  oligomers that are mediated through PrP<sup>C</sup>. For example, no dependence in LTP impairments in studies of Calella *et al.* [11] may be a result of low levels of A $\beta$  dimers in young aged Tg-mice. A protective effect of PrP<sup>C</sup> over-expression against intracerebroventricular injection of A $\beta$  may be a result of a low concentration of its assemblies. It has been proven that picomolar concentrations of the A $\beta$  did not trigger Fyn activation [47]. In our opinion, if PrP<sup>C</sup> downregulates tau protein, and A $\beta$  binds to PrP<sup>C</sup>, reverting this process, it is quite possible that effects of A $\beta$  will vary due to A $\beta$  and PrP<sup>C</sup> ratio. Noteworthy, PrP<sup>C</sup> is not only connected in aetiology of AD by its direct interactions with A $\beta$ , but also due to its negative modulation of BACE1 activity. PrP<sup>C</sup> declines with age, and is decreased in sporadic AD, but there are no alterations in familial AD cases. In sporadic

AD, the PrP<sup>c</sup> level is inversely correlated with BACE1 activity, A $\beta$  load, soluble A $\beta$ , and insoluble A $\beta$ . It is also inversely correlated with the stage of disease, as indicated by Braak tangle stages, distinguished according to the distribution of the tau pathology, especially the neurofibrillary tangles. Authors of this study point out that a decreased level of PrP<sup>c</sup> results in a decreased zinc uptake within the synapses. Such condition results in an elevated synaptic zinc level, which favours binding A $\beta$  oligomers to the NMDA receptors, and mediates the excitotoxicity [86]. In our opinion, it is more probable that an inverse correlation with Braak stage assessed tauopathy was caused by the elevation of A $\beta$  and PrP<sup>c</sup> ratio, and its direct impact on tau expression. A biophysical examination of recombinant PrP revealed that this protein represents a unique intrinsic feature to form multiple non-native isoforms rich in  $\beta$ -sheets, which may result in a large spectrum of PrP<sup>c</sup> *in vivo*. It has been proved that in uninfected human brains PrP is presented also in the form of oligomers, and even large aggregates, called insoluble PrP<sup>c</sup> (iPrP<sup>c</sup>) which stand for ~5-25% of total PrP. It is proposed that if soluble PrP<sup>c</sup> can bind to soluble A $\beta$ <sub>42</sub>, also iPrP<sup>c</sup> will bind insoluble A $\beta$ , modulating its deposition [91]. It is therefore consistent with Takahashi *et al.*'s findings [82] (confirming the prior report of Ferrer *et al.* [27]) indicating that PrP<sup>c</sup> is present in amyloid deposits. Many other related issues remain seemingly untouched. For example, amyloid A $\beta$  deposits are formed within vessels and amyloid angiopathy is not only limited to arteries, but also affects veins [60]. It is interesting whether there is also an interaction between A $\beta$  and PrP<sup>c</sup> in this compartment of brain tissue. To summarize, a growing number of research indicate new possible functions of PrP<sup>c</sup> in the field of cognition, both in its physiological and pathophysiological aspects. Considering new data, we are compelled to stronger appreciate the role of soluble forms of A $\beta$  in the pathomechanism of AD, which possibly even prevails over its insoluble deposits. This may be regarded as a "paradigm shift" in an understanding of cognitive decline in AD. Despite the remaining controversies, recent findings prompt us to consider that roads of PrP<sup>c</sup> and A $\beta$  meet at the point of tauopathy and moreover, formation of PrP<sup>c</sup>-A $\beta$  complexes may result in the consumption of PrP<sup>c</sup>, what modulates A $\beta$  neurotoxicity, possibly depriving nervous tissue of the neuroprotective function of PrP<sup>c</sup>.

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